METHODS

Human Study Cohort

The study design for the CDC AVRP human clinical trial has been reported in detail previously (1). A sub-set of 359 participants (23.0%) of the main AVRP study was consented separately to participate in the COP substudy. The study was sponsored by CDC under an Investigational New Drug application and was approved by the human investigations committees at participating clinical sites and at CDC. The study was registered at www.clinicaltrials.gov, registry number NCT00119067.

Six lots of vaccine were used over the course of the study: FAV063, FAV074, FAV079, FAV087, FAV107, and FAV113. Placebo injections were saline (0.9% wt/vol NaCl, Abbott Laboratories, Chicago, IL) Vaccination and control group schedules are provided in Table 1 (1). Study participants were randomized into one of 6 study groups to receive a 3-dose intramuscular (IM) priming schedule (0, 1, 6 Months; 3-IM) with a single booster at 42 months (4-IM); 3-IM priming with boosters at 30 and 42 months (5-IM); 3-IM priming with boosters at 12, 18, 30 and 42 months (7-IM); the 1970 licensed series of 6 doses (0, 0.5, 1, 6, 12, 18 months) and two annual boosters (30, 42 months) administered either subcutaneous (SQ) (8-SQ) or IM (8-IM); or saline control at all eight time-points. All study participants that were according to protocol (ATP) for immunogenicity received eight injections within specific time periods; saline injections were given at time points where AVA was omitted (Table 1) (1). For all SQ injections a 5/8 inch, 25-gauge needle was used for injection. For IM injections a 1-1.5 inch, 23- or 25-gauge needle was used depending on participant sex and weight.

As with the noninferiority analyses reported previously, data from all main study ATP participants were used in the analysis of human anti-PA IgG antibody responses. TNA testing was completed for all COP substudy participants and an additional 30% random selection of samples from the total study cohort for a total of 48% of all available serum samples (1). Additional humoral and CMI analyses were completed for the COP substudy. For cell-based assays, all available samples from COP substudy participants were analyzed ATP. Group sizes for humoral and CMI response analyses are provided in Tables S1 and S2. The 5 vaccination study groups were analyzed separately except that 7-IM, 5-IM and 4-IM groups were combined for time points where their schedules were the same (up to and including Month 12) or as otherwise specified in the text (1, 2). All immunological testing was blinded until the end of the study.

Anti-PA IgG Subclass Analyses

As reported previously for analogous studies in NHP, assays for all four IgG subclasses were run when the total anti-PA IgG was ≥5 μg/mL (3). Relative subclass proportions were determined in a sample when the total anti-PA IgG was ≥12.5 μg/mL, all four subclasses were evaluated and at least one IgG

subclass was detected. Detection and quantification of each of the anti-PA IgG subclasses were done in separate assays using anti-human specific monoclonal antibodies for each of the four human IgG subclasses (mouse anti-human IgG_1; Zymed Laboratories Inc., South San Francisco, CA, 05-3300; mouse anti-human IgG_2; Novus Biologicals, Littleton CO, #ab1933; mouse anti-human IgG_3; Novus Biologicals, #ab1928; and mouse anti-human IgG4; Accurate Chemical Company, Westbury, NY, #YNMAHIgG1-4SET). The conjugate HRPO-labeled sheep anti-mouse, gamma chain IgG (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, #515-035-062) was used for IgG1; and HRPO-labeled donkey anti-mouse, gamma chain IgG (Jackson ImmunoResearch Laboratories, Inc., #715-035-150) was used for IgG2, 3, and 4. The anti-PA IgG subclass concentrations were calculated in ng/mL by interpolation to an AVR414 calibration curve using a four-parameter logistic-log model (4-PL) and SoftMax Pro software (Version 4.3)(4). The LOD for IgG subclasses were 0.019, 0.081, 0.003 and 0.026 µg /mL for IgG1, IgG2, IgG3 and IgG4 respectively.

Statistical Analyses

To enhance analytical resolution, all reportable values were included in the statistical analysis. For the TNA and anti-PA IgG ELISA assays values ≤LLOQ were assigned one half the LLOQ (5). ANOVA models were used to compare the average response within each AVA study group to the saline control group and to evaluate dose-response trends. All ANOVA models were fitted separately to each reportable value at each time point using the SAS Version 9.3.1 GLM, MIXED, PROBIT, and LOGISTIC procedures. The significance level was 0.05, with adjustments for multiple comparisons.

Avidity and IgG subclass assays were run if the total anti-PA IgG was ≥5 µg/mL. IgG subclass values were set to one half the LOD when the total anti-PA IgG was detectable but <5 µg/mL. If an IgG subclass assay was performed and the IgG subclass level was <LOD, the reportable value was also set to one half the LOD. If there was insufficient sample to run some or all of the subclass assays then the subclass IgG values that were not measured were treated as missing values and not included in the analysis. For statistical analysis of the avidity assay, all values >0 were included and zero values were substituted with an arbitrary nominal value less than the smallest measured value. Tukey's multiple comparison procedure was used to compare the average AI in each vaccine dilution group to the other groups at an overall 0.05 level of significance within each set of comparisons at each time point. All valid results were included in the analysis. No attempt was made to impute missing values. For some analyses the data for the IM administered vaccine groups receiving the same schedule (4-IM, 5-IM and 7-IM up to and including Month 12; 4-IM and 5-IM for Months 13 and 18; Table 1) were combined as stated in the text.

Linear models were used to establish the correlation between quantity of anti-PA IgG antibodies and TNA and to determine whether the relationship varied with vaccine route of administration, number of doses and time of administration. All control subjects and all time points prior to two months were excluded from the analysis, as the majority of measurements were below the quantification limits for both

assays. Post-immunization blood draws collected for immune response kinetics were also excluded. At other time points, individual samples were excluded from the analysis when either the TNA ED50 or total anti-PA IgG measured by ELISA was below the assay quantification limit.

The modeling was performed in two stages. Initially, the following linear model was fitted to the data:

$$\text{Log}(Y_{ijk}) = \beta_0 + \alpha_i + \gamma_j + (\beta_1 + \tau_i + \upsilon_j + \eta_{ij})^* \log(x_{ijk})$$

where $\log(Y_{ijk})$ is the observed base-10 logarithm-transformed TNA ED50 for the k^{th} subject that received the i^{th} treatment (vaccine dose or human study arm) at the j^{th} time, β_0 is the intercept of the regression line, α_i is the effect of the ith treatment group on the intercept, γ_j is the effect of the j^{th} time on the intercept, $\log(x_{ijk})$ is the observed base-10 logarithm-transformed total anti-PA IgG measured by ELISA for the k^{th} subject that received the i^{th} treatment at the j^{th} time, β_1 is the slope of the regression line relating log total anti-PA IgG measured by ELISA to log TNA ED50, τ_i, υ_j, and η_{ij} are adjustments to the slope based on treatment group and time. The model also included a random subject effect to account for repeated measurements on the same subjects, and an error term to account for random variation not explained by the model.

Evaluation of the initial model indicated that the effects of treatment group and time were significant. For ease of interpretation, the final models were fitted separately at each time point and consequently the random subject effect was not required. An error term to account for random variation not explained by the model was included in the final models. The final regression model used to relate log10 TNA ED50 to log10 anti-PA IgG concentration $\log(Y_{ijk}) = \beta_0 + \alpha_i + \beta_1^*\log(x_{ijk})$ that included treatment group-specific intercepts and an overall slope relating anti-PA IgG to ED50. $\text{Log}(Y_{ijk})$ is the observed base-10 logarithm-transformed TNA ED50 for the k^{th} subject that received the i^{th} treatment (study group) at the j^{th} time, β_0 is the intercept of the regression line, α_i is the effect of the ith treatment group on the intercept, $\log(x_{ijk})$ is the observed base-10 logarithm-transformed (log10) total anti-PA IgG measured by ELISA for the k^{th} subject that received the i^{th} treatment at the j^{th} time, β_1 is the slope of the regression line relating log10 total anti-PA IgG log10 TNA ED50. The model also included an error term to account for random variation. All models were fitted using the SAS Version 9.1.3 MIXED procedure. Tukey's multiple comparison procedure was used to compare the intercepts for each study group to the control arm and to the other study groups at an overall 0.05 level of significance within each set of comparisons for each parameter at each time point.

DATA

Anti-PA IgG Responses

The individual group anti-PA IgG data are presented in Figure S1A and Table S6. The onset of a statistically significant anti-PA IgG response was detectable at 1 month after the first vaccination and sustained at significant levels for all AVA dose schedules for the duration of the 43 month study. At Month 1 the 8-SQ [52.6 µg/mL (95%CI 44.33, 62.39)] and 8-IM [30.6 µg/mL (95%CI 25.08, 37.4)] groups that had received vaccinations at 0 and 0.5 months produced significantly higher levels of anti-PA IgG than the 7-IM [2.1 µg/mL, (95%CI 1.8, 2.4)], 5-IM [1.6 µg/mL, (95%CI 1.4, 1.9)], or 4-IM [1.8 µg/mL, (95%CI 1.6, 2.1)] which were detectable and significantly different from controls (Table S6). All groups received a vaccination at Month 1 that stimulated an approximately 2-fold increase in anti-PA IgG by Month 2 in 8-SQ [100.7 µg/mL, (95%CI 90.1, 112.6)] and 8-IM [88.3µg/mL, (95%CI 78.0, 100.0)]; and a >25-fold increase in 7-IM [56.0 µg/mL, (95%CI 49.2, 63.7)], 5-IM [43.2 µg/mL, (95%CI 37.1, 50.3)], and 4-IM [50.4 µg/mL, (95%CI 43.9, 57.9)]. The additional dose at Month 0.5 gave detectable anti-PA IgG levels at an earlier time point and the difference in magnitude remained evident at Month 2. The impact of the Month 0.5 dose therefore has important implications for post-exposure prophylaxis use of AVA when rapid and high antibody responses are considered beneficial (6).

At Month 7, 4 weeks after administration of the Month 6 dose, the anti-PA IgG levels were statistically noninferior and not significantly different in any of the vaccination groups (range 196.2-241.1 µg/mL), indicating that priming was complete and equivalent for both the 3-dose (4-IM, 5-IM and 7-IM) and 4-dose (8-SQ and 8-IM) priming schedules (Table S6) (1). Anti-PA IgG levels subsequently declined in all groups with no significant differences at Month 12 (range 35.2-44.1 µg/mL). Study groups receiving a Month 12 booster (7-IM, 8-IM and 8-SQ) demonstrated an anamnestic response at Month 13 with the 8-IM group achieving statistically superior responses compared to 8-SQ; 7-IM [268.4 µg/mL, (95%CI 239.9, 300.3)], 8-IM [295.5 µg/mL, (95%CI 266.9, 327.0)], 8-SQ [216.3 µg/mL, (95%CI 196.6, 238.0)]. At the same 13 month time point the 5-IM and 4-IM groups had declined to 29.2, (95%CI 24.3, 35.0) and 30.2 µg/mL (95%CI 25.4, 35.8), respectively, and continued to decline to 15.1, (95%CI 12.4, 18.4) and 15.2 µg/mL (95%CI 12.7, 18.2) by Month 18 in the absence of boosters. These Month 18 levels were the lowest responses for the 5-IM group during the study. Responses in the Month 12 booster groups also declined by Month 18 in the 7-IM [51.8 µg/mL, (95%CI 45.3, 59.3)], 8-IM [55.1 µg/mL, (95%CI 48.9, 62.1)] and 8-SQ [46.0 µg/mL, (95%CI 41.2, 51.4)] which were not significantly different.

At Month 18, all groups except 4-IM received a booster. Anti-PA IgG levels in all booster groups rose significantly by Month 19. Responses were highest in the 5-IM group that received only one booster with an interval of 12 months; 5-IM [301.8 µg/mL, (95%CI 264.3, 344.5)]; 7-IM [245.5 µg/mL, (95%CI 217.3, 277.4)]; 8-IM [281.7 µg/mL, (95%CI 251.6, 315.4)] and 8-SQ [198.6 µg/mL, (95%CI 176.3,

223.8)]. The 5-IM and 8-IM group responses were significantly higher and statistically superior compared to 8-SQ (1).

Month 30 anti-PA IgG levels were the lowest (trough) values for study groups 7-IM, 8-IM and 8-SQ; 7-IM [30.3 µg/mL, (95%CI 26.4, 34.9)]; 8-IM [34.2 µg/mL, (95%CI 30.0, 38.9)] and 8-SQ [28.7 µg/mL, (95%CI 25.4, 32.4)]. At Month 30, all vaccination groups except 4-IM and 5-IM received a booster. Anti-PA IgG levels in all booster groups increased significantly by Month 31. Responses were highest in the 8-IM group and were the maximum point estimate for this group during the entire study: 8-IM [359.2 µg/mL, (95%CI 322.4, 400.3)]. The other two booster group responses in order of magnitude were 7-IM [309.6 µg/mL, (95%CI 276.8, 346.3)] and 8-SQ [250.9 µg/mL, (95%CI 224.0, 281.0)]. The 8-IM group response was statistically superior to the 8-SQ response, but not statistically significantly different from 7-IM. Responses in the unboosted groups remained relatively stable in the same interval; 4-IM Month 30 [8.6 µg/mL, (95%CI 7.1, 10.4)] and Month 31 [5.6 µg/mL, (95%CI 4.6, 6.8)]; 5-IM Month 30 [35.8 µg/mL, (95%CI 30.2, 42.5)] and Month 31 [33.8 µg/mL, (95%CI 28.5, 40.2)].

Month 42 anti-PA IgG levels were the trough values for the 4-IM group that had received no boosters since completion of priming at Month 6. In this group, 66.5% (107/161) had quantifiable (≥LLOQ) anti-PA IgG: 4-IM [5.6, (95%CI 4.6, 6.8)] (Table S6). The other group responses in order of increasing magnitude were 5-IM [22.0 µg/mL, (95%CI 18.4, 26.2)]; 8-SQ [38.9 µg/mL, (95%CI 34.4, 43.9)]; 7-IM [42.1 µg/mL, (95%CI 36.7, 48.4)]; 8-IM [50.3 µg/mL, (95%CI 44.3, 57.0)]. Responses were not significantly different between the 7-IM, 8-IM and 8-SQ groups and these groups were significantly higher than 4-IM and 5-IM. The 5-IM responses were significantly higher than 4-IM.

All groups received their final booster at Month 42 and final responses were determined at Month 43 (1). The interval between boosters was 36 months for the 4-IM group, 24 months for 5-IM and 12 months for each of 7-IM, 8-IM and 8-SQ. Responses at Month 43 were significantly higher in all of the IM groups compared to the 8-SQ group [219.2 µg/mL, (95%CI 195.3, 246.0)]. The 4-IM group with a 36 month interval in vaccinations had the highest anti-PA IgG response: 4-IM [438.6 µg/mL, (95%CI 373.5, 514.9)], which was more than twice the response in the comparator 8-SQ group. The other group responses in order of increasing magnitude were 7-IM [298.6 µg/mL, (95%CI 263.1, 339.0)], 5-IM [314.7 µg/mL, (95%CI 274.1, 361.3)] and 8-IM [340.7 µg/mL, (95%CI 306.5, 378.8)]. Responses were not significantly different between the 5-IM, 7-IM and 8-IM groups (Wright *et al.*, 2014). The 4-IM response was significantly (more than 2-fold) higher than all other groups and statistically superior. At the completion of the study there was an anti-PA IgG response hierarchy of 4-IM > 5-IM > 7-IM and 8-IM > 8-SQ; fewer boosters with greater intervals between injections generated higher anti-PA IgG levels.

Lethal Toxin Neutralization Activity

Forty eight percent (48%) of all available human serum samples were evaluated for TNA (1). Data are presented in Table S7 and Figure S1B. TNA at Month 1 for the two study groups that received

vaccinations at 0 and 0.5 months, the 8-SQ [ED50=82.5, (95%CI 60.2, 112.9)] and 8-IM [(ED50=64.4, (95%CI 47.3, 87.7)], were significantly increased compared to all other groups, which were detectable but <LOD (Table S7). The third vaccination for these groups at Month 1 stimulated a >2-fold increase in TNA by Month 2 in 8-SQ [ED50=231.1, (95%CI 190.9, 279.8)] and >3-fold in 8-IM [ED50=247.3, (95%CI 202.5, 302.0)]. In contrast to the anti-PA IgG responses, the impact on TNA of omitting the vaccination at 0.5 months was less pronounced at Month 2. Groups receiving their second vaccination at Month 1 demonstrated a >20-fold TNA increase by Month 2: 7-IM [ED50=185.4, (95%CI 151.1, 227.3)], 5-IM [ED50=130.7, (95%CI 101.0, 169.0)], and 4-IM [ED50=158.3, (95%CI 122.5, 204.6)]. All groups demonstrated a decline in TNA between Months 2 and 6, with only the 8-SQ and 8-IM groups maintaining TNA significantly >LLOQ.

At Month 7, 4 weeks after administration of the Month 6 dose, the TNA levels (ED50 range 1297.3-1630.0) were not significantly different in any of the vaccination groups, indicating that priming was complete and equivalent for both the 4-dose (8-SQ and 8-IM) and 3-dose (4-IM, 5-IM and 7-IM) priming schedules. As previously reported, the Month 7 TNA responses were also statistically noninferior between groups (1). TNA subsequently declined in all groups with no significant differences at Month 12 (ED50 range 225.0-283.9). Study groups receiving a Month 12 (7-IM, 8-IM and 8-SQ) demonstrated an anamnestic response at Month 13. The 8-IM group was significantly higher than the 8-SQ group: 8-IM [ED50=1932.7, (95%CI 1672.9, 2232.8)], 8-SQ [ED50=1355.5, (95%CI 11549.6, 1584.5), 7-IM [ED50=1778.9, (95%CI 1510.9, 2094.4). At the same 13 month time point the TNA in non-boosted 4-IM and 5-IM groups had declined to ED50=165.5 (95%CI 125.6, 218.0) and ED50=197.1 (95%CI 149.5, 259.8), respectively, and continued to decline to ED50=88.7 (95%CI 65.5, 120.0) and ED50=96.3 (95%CI 69.9, 132.7) by Month 18. As with anti-PA IgG, these were the lowest TNA post-priming response levels for the 5-IM group during the study. Responses in the Month 12 booster groups also declined significantly by Month 18; 7-IM [ED50=320.4, (95%CI 248.6, 413.0)]; 8-IM [ED50=325.1, (95%CI 262.7, 402.3)] and 8-SQ [ED50=273.0, (95%CI 222.5, 334.8)] which were not significantly different from each other, but were significantly higher than the groups that did not receive a boost.

At Month 18, all groups except 4-IM received a booster. TNA levels in all booster groups rose significantly by Month 19. Responses were highest in the 5-IM group that received only one booster with an interval of 12 months; 5-IM [ED50=1963.8, (95%CI 1625.8, 2372.0)]; 7-IM [ED50=1343.2, (95%CI 1120.8, 1609.8)]; 8-IM [ED50=1446.5, (95%CI 1203.1, 1739.0)] and 8-SQ [ED50=1137.0, (95%CI 945.6, 1367.0)]. Of the groups that received a booster, the 5-IM group responses were significantly higher than 8-SQ and 7-IM.

Month 30 TNA were the trough values for study groups 7-IM, 8-IM and 8-SQ; 7-IM [ED50=201.0, (95%CI 158.8, 254.5)]; 8-IM [ED50=206.9, (95%CI 171.2, 250.2)] and 8-SQ [ED50=171.7, (95%CI 140.0, 210.6)]. At Month 30, all groups except 4-IM and 5-IM received a booster. TNA levels in all

booster groups increased significantly by Month 31. Responses were highest in the 8-IM group and were the maximum point estimate for this group during the entire study: 8-IM [ED50=1743.5, (95%CI 1446.7, 2101.2)]. The other two booster group responses in order of magnitude were 8-SQ [ED50=1116.9, (95%CI 936.2, 1332.6)] and 7-IM [ED50=1544.4, (95%CI 1257.5, 1896.7)]. The 8-IM group was significantly higher than the 8-SQ group. Responses in the unboosted groups remained relatively stable in the same interval with only small declines; 4-IM Month 30 [ED50=56.2, (95%CI 40.0, 79.0)] and Month 31 [ED50=52.4, (95%CI 38.5, 71.5)]; 5-IM Month 30 [ED50=300.4, (95%CI 242.8, 371.6)] and Month 31 [ED50=207.4, (95%CI 155.2, 277.0)].

Month 42 TNA levels were the trough values for the 4-IM group that had received no boosters since completion of priming at Month 6. In this group at this time point 81.0% of participants (57/70) had quantifiable TNA (≥LLOQ): 4-IM [ED50=36.35, (95%CI 27.1, 48.8)]. The other group responses in order of increasing magnitude were 5-IM [ED50=168.5, (95%CI 131.8, 215.3)], 8-SQ [ED50=213.6, (95%CI 167.1, 273.0)], 7-IM [ED50=217.6, (95%CI 169.2, 279.9)] and 8-IM [ED50=298.9, (95%CI 245.7, 363.6)]. Responses were not significantly different between the 7-IM, 8-IM and 8-SQ groups. The 8-IM group was significantly higher than 5-IM. The 4-IM responses were significantly lower than all other groups.

All groups received their final booster at Month 42 and final responses were determined at Month 43 (Wright *et al.*, 2014). The interval between boosters was 36 months for the 4-IM group, 24 months for 5-IM and 12 months for each of 7-IM, 8-IM and 8-SQ. The 8-SQ group responses [ED50=1005.68, (95%CI 822.38, 1229.84)] at Month 43 were significantly lower compared to the 4-IM and 5-IM groups. The 4-IM group with a 36 month interval in vaccinations had the highest TNA response: 4-IM [ED50=2853.7, (95%CI 2203.7, 3695.3)]. The other group responses in order of increasing magnitude were 7-IM [ED50=1469.5, (95%CI 1167.8, 1849.1)], 8-IM [ED50=1540.3, (95%CI 1274.6, 1861.4)] and 5-IM [ED50=1918.7, (95%CI 1640.3, 2244.4)]. Responses were not significantly different between the 5-IM, 7-IM and 8-IM groups. Except for 7-IM, all IM group responses were significantly higher than the 8-SQ. The 4-IM response was significantly higher than all other groups. At the completion of the study there was a TNA response hierarchy of 4-IM > 5-IM > 8-IM ≥ 7-IM > 8-SQ. Fewer boosters with greater intervals between injections generated higher TNA levels at Month 42 (Table S7).

TABLE S1. Intention to Treat (ITT) and According to Protocol (ATP) Group Sample Sizes for Anti-PA IgG and TNA Immune Response Analyses

Month	*COP Anti-PA IgG ELISA Samples ITT	COP Anti-PA IgG ELISA Samples ATP	Percentage of COP Anti-PA IgG ELISA ATP	Anti-PA IgG ELISA Samples ATP	Percentage of Anti-PA IgG ELISA ATP	TNA ED50 Samples ITT	TNA ED50 Samples ATP	Percentage of TNA ED50 Samples ATP
0	359	359	100	1563	100	704	704	100
0.5	343	343	100	343	100	343	343	100
1	351	335	95.44	1452	92.90	698	666	95.42
2	347	327	94.24	1438	92.00	707	664	93.92
6	344	308	89.53	1344	85.99	723	650	89.5
7	339	301	88.79	1319	84.39	686	614	89.50
12	333	290	87.09	1264	80.87	664	591	89.01
13	327	283	86.54	1230	78.69	676	593	87.72
18	319	264	82.76	1162	74.34	616	516	83.77
19	317	259	81.70	1137	72.74	620	514	82.90
30	294	234	79.59	1044	66.79	590	466	78.98
31	290	227	78.28	1011	64.68	569	450	79.09
42	264	201	76.14	912	58.35	554	422	76.17
43	260	194	74.61	891	57.01	533	398	74.67

*COP – Correlates of Protection substudy participants including control groups.

TABLE S2. Group sizes ATP for COP substudy cellular immune response analyses

	Sample Sizes By Assay, Group and Time Point																							
	Lymphocyte Stimulation Indices (SI)						IL-4 ELISpot						IFN-γ ELISpot						Cytokine mRNA Level Analyses					
Time Point (Months)	4-IM	5-IM	7-IM	8-IM	8-SQ	C	4-IM	5-IM	7-IM	8-IM	8-SQ	C	4-IM	5-IM	7-IM	8-IM	8-SQ	C	4-IM	5-IM	7-IM	8-IM	8-SQ	C
0	60	60	56	58	60	61	57	57	51	59	57	59	59	57	51	58	55	59	58	59	55	58	58	61
0.5	54	55	51	51	51	54	53	56	49	49	49	55	53	57	48	50	48	55	53	56	53	48	51	53
1	57	59	51	52	55	59	53	58	51	52	54	55	48	56	47	47	53	52	56	56	51	48	53	57
2	52	56	50	51	52	55	53	58	48	51	51	53	49	57	43	51	46	53	55	55	48	51	51	57
6	50	53	47	50	47	54	48	48	48	47	45	50	45	47	46	45	45	50	43	49	48	47	43	50
7	49	48	50	50	46	52	49	44	47	46	43	44	49	44	45	44	43	44	44	46	48	48	41	48
12	47	48	47	46	42	52	41	42	41	40	37	41	42	44	44	37	36	44	40	39	41	43	39	45
13	47	46	47	45	42	46	41	41	40	35	39	49	40	40	44	42	36	47	39	41	41	42	36	42
18	44	38	41	42	42	50	39	37	37	36	38	49	37	32	31	34	38	46	23	19	27	22	24	26
19	42	38	41	37	42	48	37	36	42	37	35	44	39	34	41	33	36	44	20	17	23	16	20	25
30	41	33	37	38	38	40	36	30	35	36	36	41	36	29	35	34	36	34	N/S	N/S	N/S	N/S	N/S	N/S
31	39	30	35	39	38	41	37	30	32	35	34	39	32	30	30	34	33	37	N/S	N/S	N/S	N/S	N/S	N/S
42	34	29	23	35	31	37	34	31	26	35	33	38	31	29	24	34	30	37	N/S	N/S	N/S	N/S	N/S	N/S
43	33	30	23	32	29	33	33	30	24	34	30	35	30	28	23	31	29	31	N/S	N/S	N/S	N/S	N/S	N/S

TABLE S2 (continued). Group sizes ATP for COP substudy immune response analyses

	Sample Sizes By Assay, Group and Time Point																	
		Anti-PA IgG Avidity Indices (AI)						IgG Subclass Distributions						*Memory B Cell Analyses					
		4-IM	5-IM	7-IM	8-IM	8-SQ	C	4-IM	5-IM	7-IM	8-IM	8-SQ	C	4-IM	5-IM	7-IM	8-IM	8-SQ	C
Time Point (Months)	**0**	0	0	0	0	0	0	10	8	4	9	12	10	5	10	7	7	7	8
	0.5	0	0	1	0	0	0	8	7	5	7	5	9	3	7	5	5	6	4
	1	2	1	2	27	32	0	10	8	7	15	20	11	6	6	6	7	6	6
	2	39	37	32	41	36	0	24	24	17	21	19	14	7	8	8	5	7	6
	6	3	1	5	13	8	0	28	30	30	29	25	27	7	7	8	8	6	7
	7	50	49	49	50	45	0	42	39	40	41	36	34	37	40	39	38	36	42
	12	37	37	37	43	39	0	42	46	48	46	41	37	9	4	6	8	6	9
	13	36	33	48	47	44	0	42	41	48	47	42	37	6	4	5	6	5	7
	18	25	22	39	42	37	2	32	28	39	43	39	20	24	29	28	27	34	34
	19	22	40	42	42	43	0	26	40	42	42	43	13	6	2	5	3	5	8
	30	18	30	30	36	31	0	20	30	30	37	32	2	8	3	9	6	6	6
	31	16	28	35	37	37	0	17	28	35	38	38	3	8	1	7	5	6	6
	42	11	24	23	35	27	0	12	25	23	35	28	0	26	21	21	24	24	33
	43	34	31	25	34	32	0	34	31	25	35	32	0	5	4	2	6	8	5

N/S – No sample tested. *For Memory B Cell analyses, due to small samples sizes, groups 7-IM, 5-IM, and 4-IM were combined up to month 12 (754-IM), and groups 5-IM and 4-IM were combined for months 13 and 18 (54-IM).

TABLE S3. Sample sizes for human kinetics study participants at each day post-injection at Month 6

Study Group	Days Post Injection													
	3	4	5	6	7	8	9	10	11	12	13	14	15	Total
8-SQ	18	7	23	19	36	19	19	4	10	13	12	33	6	219
8-IM	17	14	25	19	44	14	21	2	3	8	14	30	10	221
754-IM	49	36	66	67	126	49	49	10	18	38	41	62	29	640
Control	16	11	23	22	36	29	15	5	10	12	8	32	5	224
Total	100	68	137	127	242	111	104	21	41	71	75	157	50	1303

Data represent the number of participants at each day post-injection within each study group. For kinetics analyses at Month 6, due to small samples sizes, Groups 7-IM, 5-IM, and 4-IM were combined (754-IM).

TABLE S4. Sample sizes for human kinetics study participants at each day post-injection at Month 30

Study Group	Days Post Injection													
	3	4	5	6	7	8	9	10	11	12	13	14	15	Total
8-SQ	15	11	23	8	40	15	8	4	0	13	12	16	5	170
8-IM	15	9	19	11	34	21	8	3	5	15	12	20	4	176
7-IM	15	9	12	23	29	13	12	6	8	10	10	12	8	167
5-IM	6	16	17	16	26	14	7	3	9	4	8	21	5	152
4-IM	17	13	17	10	36	18	8	3	6	10	14	25	2	179
Control	12	6	16	19	32	21	6	2	7	11	7	16	4	159
Total	80	64	104	87	197	102	49	21	35	63	63	110	28	1003

Data represent the number of participants at each day post-injection within each study group.

TABLE S5. Sample sizes for human kinetics study participants at each day post-injection at Month 42

Study Group	Days Post Injection													
	3	4	5	6	7	8	9	10	11	12	13	14	15	Total
8-SQ	6	6	21	18	25	12	10	2	7	9	12	12	3	143
8-IM	15	8	17	13	36	8	8	3	4	13	10	18	3	156
7-IM	10	5	18	11	35	12	5	4	4	8	7	14	7	140
5-IM	10	8	13	16	38	12	4	3	7	8	9	13	2	143
4-IM	14	11	13	12	31	15	4	4	4	10	10	25	3	156
Control	12	8	15	17	24	9	12	2	3	10	16	14	0	142
Total	67	46	97	87	189	68	43	18	29	58	64	96	18	880

Data represent the number of participants at each day post-injection within each study group.

TABLE S6. Geometric mean anti-PA IgG concentrations by study group ATP

Time Point (Months)	Human Study Group Anti-PA IgG (GMC µg/mL) (95% CI)						
	8-SQ	8-IM	7-IM	5-IM	4-IM	754-IM	Controls
0.5	1.27 (1.02, 1.59) N=58	1.22 (1, 1.49) N=56	1.12 (0.92, 1.35) N=55	1.08 (0.93, 1.26) N=58	1.08 (0.94, 1.25) N=59	1.09 (1.00, 1.20) N=172	0.88 (0.84, 0.93) N=57
1	52.59 (44.33, 62.39) N=242	30.62 (25.08, 37.4) N=241	2.06 (1.77, 2.4) N=239	1.64 (1.44, 1.87) N=240	1.84 (1.59, 2.13) N=244	1.84 (1.69, 2.00) N=723	0.91 (0.87, 0.94) N=246
2	100.7 (90.05, 112.62) N=239	88.28 (77.95, 99.98) N=238	55.99 (49.2, 63.7) N=238	43.22 (37.14, 50.31) N=238	50.40 (43.86, 57.91) N=240	49.59 (45.74, 53.78) N=716	0.93 (0.88, 0.98) N=245
6	9.93 (8.76, 11.26) N=226	8.64 (7.47, 10) N=226	4.23 (3.68, 4.86) N=223	3.40 (2.94, 3.92) N=214	3.53 (3.04, 4.10) N=225	3.70 (3.41, 4.03) N=662	0.90 (0.87, 0.94) N=230
7	214.11 (191.16, 239.82) N=222	241.14 (213.91, 271.84) N=223	240.22 (210.36, 274.33) N=219	196.21 (168.27, 228.80) N=206	229.52 (198.76, 265.03) N=224	221.76 (204.18, 240.86) N=649	0.93 (0.87, 0.98) N=225
12	43.74 (38.23, 50.03) N=210	44.12 (38.1, 51.08) N=214	40.43 (34.3, 47.64) N=211	35.15 (29.35, 42.09) N=197	36.10 (30.54, 42.66) N=215	37.19 (33.73, 41.02) N=623	0.91 (0.87, 0.95) N=217
13	216.31 (196.64, 237.95) N=205	295.46 (266.93, 327.04) N=210	268.44 (239.93, 300.34) N=206	29.17 (24.33, 34.98) N=189	30.15 (25.36, 35.84) N=212	NA	0.92 (0.87, 0.96) N=208
18	46.04 (41.23, 51.42) N=194	55.09 (48.85, 62.14) N=196	51.82 (45.26, 59.32) N=194	15.13 (12.42, 18.42) N=179	15.20 (12.67, 18.23) N=201	NA	0.90 (0.86, 0.94) N=198
19	198.75 (176.51, 223.8) N=191	280.34 (250.77, 313.39) N=189	244.97 (216.91, 276.65) N=193	299.05 (262.17, 341.11) N=176	14.45 (12.11, 17.23) N=195	NA	0.90 (0.86, 0.93) N=193
30	28.72 (25.43, 32.44) N=176	34.18 (30.03, 38.9) N=178	30.34 (26.35, 34.93) N=179	35.81 (30.21, 42.45) N=162	8.58 (7.09, 10.37) N=182	NA	0.89 (0.86, 0.92) N=167
31	250.87 (223.97, 281) N=170	359.24 (322.41, 400.28) N=173	309.61 (276.84, 346.26) N=171	33.81 (28.45, 40.17) N=155	8.06 (6.65, 9.76) N=180	NA	0.89 (0.85, 0.92) N=162
42	38.86 (34.36, 43.94) N=151	50.25 (44.3, 57) N=159	42.12 (36.67, 48.39) N=147	21.99 (18.43, 26.24) N=145	5.60 (4.63, 6.77) N=161	NA	0.95 (0.90, 1.00) N=149
43	219.23 (195.38, 245.99) N=146	340.71 (306.49, 378.75) N=157	298.64 (263.11, 338.97) N=144	314.73 (274.14, 361.33) N=143	438.54 (373.53, 514.87) N=158	NA	0.94 (0.891.00) N=143

Responses are geometric means with 95% confidence intervals. Geometric means were calculated after masking results below the LOD of 1.7 µg/mL with ½ the LOD (0.85 µg/mL). N = number of ATP individuals tested at that time point. The 754-IM column contains the results of combining the 7-IM, 5-IM, and 4-IM groups up to Month 12, during which they have received the same number of doses. NA = Not applicable.

TABLE S7. Geometric mean TNA Titers (ED50) by study group ATP

	Human Study Group TNA (GMT ED50) (95% CI)						
Time Point (Months)	8-SQ	8-IM	7-IM	5-IM	4-IM	754-IM	Controls
0.5	6.60 (5.67, 7.69) N=58	6.13 (5.49, 6.85) N=56	6.76 (5.42, 8.43) N=55	5.98 (5.45, 6.57) N=58	5.68 (5.32, 6.06) N=59	6.11 (5.64, 6.62) N=172	5.59 (5.41, 5.78) N=57
1	82.47 (60.24, 112.91) N=105	64.41 (47.32, 87.69) N=108	7.99 (6.79, 9.41) N=116	6.49 (5.80, 7.26) N=113	7.33 (6.18, 8.69) N=108	7.25 (6.65, 7.90) N=337	5.56 (5.44, 5.69) N=116
2	231.09 (190.88, 279.77) N=114	247.30 (202.51, 301.98) N=104	185.36 (151.13, 227.34) N=111	130.67 (101.02, 169.01) N=106	158.31 (122.47, 204.63) N=105	156.92 (136.73, 180.09) N=322	5.56 (5.44, 5.68) N=124
6	29.71 (23.04, 38.31) N=99	32.84 (26.22, 41.13) N=124	11.01 (9.11, 13.30) N=108	9.83 (8.21, 11.76) N=99	12.85 (10.36, 15.95) N=106	11.19 (10.00, 12.53) N=313	5.73 (5.50, 5.96) N=114
7	1297.27 (1087.45, 1547.59) N=101	1629.98 (1354.30, 1961.74) N=105	1489.31 (1230.57, 1802.45) N=104	1391.89 (1104.94, 1753.35) N=92	1408.95 (1104.66, 1797.06) N=102	1431.09 (1260.65, 1624.59) N=298	5.97 (5.61, 6.34) N=110
12	224.96 (182.13, 277.86) N=93	283.88 (234.42, 343.77) N=101	220.91 (165.28, 295.25) N=99	231.11 (174.54, 306.01) N=88	231.19 (181.62, 294.27) N=105	227.63 (195.09, 265.59) N=292	5.56 (5.44, 5.68) N=105
13	1355.49 (1159.56, 1584.54) N=109	1932.66 (1672.89, 2232.77) N=103	1778.85 (1510.86, 2094.38) N=93	197.06 (149.49, 259.77) N=92	165.47 (125.57, 218.04) N=95	NA	5.50 (*) N=101
18	272.95 (222.54, 334.77) N=80	325.06 (262.65, 402.31) N=87	320.42 (248.62, 412.97) N=79	96.31 (69.90, 132.70) N=84	88.66 (65.49, 120.02) N=96	NA	5.68 (5.48, 5.89) N=90
19	1136.95 (945.62, 1366.99) N=87	1446.47 (1203.14, 1739.02) N=84	1343.22 (1120.80, 1609.76) N=100	1963.78 (1625.82, 2371.99) N=86	84.07 (61.74, 114.49) N=76	NA	5.89 (5.49, 6.32) N=81
30	171.66 (139.95, 210.55) N=75	206.94 (171.19, 250.16) N=83	201.03 (158.81, 254.47) N=79	300.36 (242.75, 371.64) N=78	56.21 (40.01, 78.99) N=71	NA	5.93 (5.43, 6.47) N=80
31	1116.92 (936.18, 1332.56) N=72	1743.51 (1446.69, 2101.23) N=72	1544.39 (1257.50, 1896.74) N=77	207.35 (155.20 277.04) N=69	52.42 (38.46, 71.46) N=80	NA	5.50 (*) N=80
42	213.58 (167.10, 272.98) N=64	298.90 (245.74, 363.57) N=73	217.63 (169.23, 279.87) N=67	168.46 (131.80, 215.32) N=72	36.35 (27.06, 48.82) N=70	NA	6.05 (5.64, 6.48) N=76
43	1005.68 (822.38, 1229.84) N=61	1540.27 (1274.60, 1861.39) N=72	1469.47 (1167.79, 1849.10) N=59	1918.72 (1640.3, 2244.4) N=69	2853.66 (2203.71, 3695.29) N=67	NA	5.77 (5.45, 6.10) N=70

Responses are geometric means with 95% confidence intervals. Geometric means were calculated after masking results below the LOD of 11 with ½ the LOD (5.5). The 754-IM column contains the results of combining the 7-IM, 5-IM, and 4-IM groups up to Month 12, during which they have received the same number of doses. N = number of ATP individuals tested at that time point.
NA = Not applicable. *All values were ≤LOD, therefore no confidence interval could be calculated.

TABLE S8. Anti-PA IgG avidity indices by study group ATP

Time Point (Month)	Study Group	Number of Observations/group (N)	N>0	Mean AI	Standard Error	Group Effect P-value	Significant Differences Between Study Groups Tukey's P-value #
1	8-SQ	32	32	0.16	0.01	0.7092	
	8-IM	27	27	0.19	0.04		
	7-IM	2	2	0.26	0		
	5-IM	1	1	0.29	-		
	4-IM	2	2	0.12	0.06		
2	8-SQ	36	36	0.2	0.01	0.7775	
	8-IM	41	41	0.2	0.01		
	7-IM	32	32	0.21	0.01		
	5-IM	37	36	0.21	0.01		
	4-IM	39	39	0.21	0.01		
6	8-SQ	8	8	0.26	0.03	0.2417	
	8-IM	13	13	0.37	0.05		
	7-IM	5	5	0.24	0.04		
	5-IM	1	1	0.22	-		
	4-IM	3	3	0.19	0.05		
7	8-SQ	45	44	0.37	0.02	0.0050*	8-IM>7-IM, 0.0251 8-IM>5-IM, 0.0426 8-IM>4-IM, 0.0334
	8-IM	50	50	0.39	0.01		
	7-IM	49	49	0.33	0.01		
	5-IM	49	49	0.34	0.01		
	4-IM	50	50	0.34	0.01		
12	8-SQ	39	38	0.35	0.02	0.1800	
	8-IM	43	42	0.39	0.02		
	7-IM	37	37	0.36	0.02		
	5-IM	37	37	0.33	0.01		
	4-IM	37	36	0.35	0.02		
13	8-SQ	44	44	0.4	0.01	0.0877	
	8-IM	47	46	0.39	0.02		
	7-IM	48	48	0.37	0.01		
	5-IM	33	33	0.33	0.02		
	4-IM	36	36	0.37	0.02		
18	8-SQ	37	37	0.39	0.02	0.0061*	8-SQ>5-IM, 0.0214 8-IM> 5-IM, 0.0265 5-IM<4-IM, 0.0124
	8-IM	42	42	0.39	0.01		
	7-IM	39	38	0.35	0.02		
	5-IM	22	22	0.29	0.02		
	4-IM	25	25	0.41	0.04		
19	8-SQ	43	42	0.41	0.02	0.1226	
	8-IM	42	41	0.44	0.02		
	7-IM	42	42	0.47	0.02		
	5-IM	40	40	0.42	0.02		
	4-IM	22	22	0.39	0.03		
30	8-SQ	31	31	0.39	0.02	0.0913	
	8-IM	36	36	0.42	0.02		
	7-IM	30	27	0.33	0.03		
	5-IM	30	28	0.38	0.03		
	4-IM	18	18	0.35	0.03		
31	8-SQ	37	36	0.45	0.02	0.1132	
	8-IM	37	37	0.51	0.02		
	7-IM	35	34	0.46	0.03		
	5-IM	28	27	0.4	0.03		
	4-IM	16	16	0.43	0.07		

Time Point (Month)	Study Group	Number of Observations/group (N)	N>0	Mean AI	Standard Error	Group Effect P-value	Significant Differences Between Study Groups Tukey's P-value #
42	8-SQ	27	26	0.39	0.03	0.0010*	8-IM>4-IM, 0.0260 7-IM>5-IM, 0.0134 7-IM>4-IM, 0.0053
	8-IM	35	34	0.44	0.03		
	7-IM	23	23	0.48	0.04		
	5-IM	24	22	0.33	0.03		
	4-IM	11	10	0.28	0.05		
43	8-SQ	32	30	0.51	0.04	0.1596	
	8-IM	34	34	0.54	0.02		
	7-IM	25	25	0.55	0.06		
	5-IM	31	30	0.45	0.02		
	4-IM	34	34	0.45	0.03		

Tukey's multiple comparison procedure was used to compare the average AI in each vaccine dilution group to the other groups at an overall 0.05 level of significance within each set of comparisons at each time point.

Cells contain all pairwise comparisons between Groups that are significant at the 0.05 level. The format within each cell is: (1) the relationship between corresponding arm means and (2) the Tukey-adjusted p-value.

* Statistically significant at the 0.05 level of significance.

TABLE S9. PA-Specific lymphocyte stimulation indices (SI) by study group ATP

Month	Group	Number of Observations/group (N)	N>0	Geometric Mean (95% Confidence Interval)	Group Effect P-value	Tukey Adjusted P-value for Significant Differences from Controls (C)	Significant Differences Between the Groups (Relationship) Tukey's P-value #
0	8-SQ	60	60	1.16 (1.02, 1.31)	0.0625	0.6780	8-SQ>4-IM, 0.0295
	8-IM	58	58	0.98 (0.86, 1.11)		0.9964	
	7-IM	56	56	0.96 (0.84, 1.10)		0.9871	
	5-IM	60	60	0.95 (0.85, 1.06)		0.9588	
	4-IM	60	60	0.89 (0.78, 1.01)		0.6104	
	C	61	61	1.02 (0.92, 1.13)			
0.5	8-SQ	51	51	1.62 (1.30, 2.00)	0.0079*	0.0142*	
	8-IM	51	51	1.39 (1.19, 1.63)		0.3147	
	7-IM	51	51	1.63 (1.32, 2.01)		0.0112*	
	5-IM	55	55	1.5 (1.25, 1.79)		0.0750	
	4-IM	54	54	1.32 (1.13, 1.54)		0.5494	
	C	54	54	1.08 (0.96, 1.21)			
1	8-SQ	55	55	3.52 (2.71, 4.58)	<0.0001*	<0.0001*	
	8-IM	52	52	2.03 (1.49, 2.78)		0.0005*	8-SQ>8-IM, 0.0077
	7-IM	51	51	1.93 (1.52, 2.46)		0.0018*	8-SQ>7-IM, 0.0027
	5-IM	59	59	1.44 (1.21, 1.72)		0.2967	8-SQ>5-IM, <0.0001
	4-IM	57	57	1.66 (1.39, 1.99)		0.0348*	8-SQ>4-IM, <0.0001
	C	59	59	1.05 (0.92, 1.20)			
2	8-SQ	52	52	2.29 (1.85, 2.84)	<0.0001*	<0.0001*	
	8-IM	51	51	2.66 (2.10, 3.38)		<0.0001*	
	7-IM	50	50	2.57 (2.04, 3.23)		<0.0001*	
	5-IM	56	56	2.32 (1.91, 2.83)		<0.0001*	
	4-IM	52	52	2.23 (1.61, 3.10)		<0.0001*	
	C	55	55	1.04 (0.92, 1.17)			
6	8-SQ	47	47	2.12 (1.65, 2.72)	<0.0001*	0.0007*	
	8-IM	50	50	2.36 (1.86, 3.00)		<0.0001*	
	7-IM	47	47	2 (1.46, 2.75)		0.0025*	
	5-IM	53	53	2.08 (1.63, 2.65)		0.0007*	
	4-IM	50	50	2.1 (1.59, 2.77)		0.0007*	
	C	54	54	1.05 (0.93, 1.18)			
7	8-SQ	46	46	2.89 (2.05, 4.07)	<0.0001*	<0.0001*	
	8-IM	50	50	2.71 (2.05, 3.57)		<0.0001*	
	7-IM	50	50	3.05 (2.22, 4.18)		<0.0001*	
	5-IM	48	48	2.83 (2.04, 3.92)		<0.0001*	
	4-IM	49	49	2.17 (1.63, 2.89)		0.0007*	
	C	52	52	0.96 (0.85, 1.09)			
12	8-SQ	42	42	2.27 (1.60, 3.23)	0.0002*	0.0211*	
	8-IM	46	46	2.11 (1.54, 2.88)		0.0455*	
	7-IM	47	47	2.77 (1.93, 3.96)		0.0005*	
	5-IM	48	48	2.37 (1.70, 3.31)		0.0075*	
	4-IM	47	47	2.75 (1.95, 3.87)		0.0006*	
	C	52	52	1.13 (0.96, 1.32)			
13	8-SQ	42	42	2.95 (2.03, 4.30)	<0.0001*	0.0003*	
	8-IM	45	45	3.4 (2.43, 4.75)		<0.0001*	
	7-IM	47	47	3.07 (2.07, 4.57)		<0.0001*	
	5-IM	46	46	2.38 (1.73, 3.27)		0.0070*	
	4-IM	47	47	2.57 (1.81, 3.65)		0.0019*	
	C	46	46	1.07 (0.95, 1.19)			
18	8-SQ	42	42	3.97 (2.82, 5.59)	<0.0001*	<0.0001*	
	8-IM	42	42	3.02 (2.18, 4.18)		0.0003*	

Month	Group	Number of Observations/group (N)	N>0	Geometric Mean (95% Confidence Interval)	Group Effect P-value	Tukey Adjusted P-value for Significant Differences from Controls (C)	Significant Differences Between the Groups (Relationship) Tukey's P-value #
	7-IM	41	41	3.44 (2.50, 4.74)		<0.0001*	
	5-IM	38	38	2.79 (1.86, 4.16)		0.0022*	
	4-IM	44	44	2.56 (1.92, 3.41)		0.0056*	
	C	50	50	1.25 (1.08, 1.43)			
19	8-SQ	42	42	3.83 (2.58, 5.69)	<0.0001*	<0.0001*	
	8-IM	37	37	3.81 (2.63, 5.53)		<0.0001*	
	7-IM	41	41	4.46 (3.18, 6.25)		<0.0001*	
	5-IM	38	38	3.62 (2.40, 5.46)		<0.0001*	
	4-IM	42	42	2.44 (1.62, 3.67)		0.0079*	
	C	48	48	1.07 (0.89, 1.28)			
30	8-SQ	38	38	2.04 (1.46, 2.85)	0.0004*	0.0838	
	8-IM	38	38	2.47 (1.75, 3.49)		0.0058*	
	7-IM	37	37	2.45 (1.61, 3.71)		0.0073*	
	5-IM	33	33	3.07 (2.04, 4.62)		0.0002*	
	4-IM	41	41	2.13 (1.71, 2.64)		0.0422*	
	C	40	40	1.14 (1.00, 1.29)			
31	8-SQ	38	38	2.42 (1.73, 3.39)	<0.0001*	0.0019*	
	8-IM	39	39	3.01 (2.19, 4.15)		<0.0001*	
	7-IM	35	35	2.16 (1.62, 2.87)		0.0154*	
	5-IM	30	30	2.73 (1.85, 4.03)		0.0006*	
	4-IM	39	39	2.16 (1.45, 3.20)		0.0113*	
	C	41	41	1.05 (0.90, 1.22)			
42	8-SQ	31	31	1.99 (1.57, 2.51)	0.0001*	0.0305*	
	8-IM	35	35	2.35 (1.70, 3.25)		0.0015*	
	7-IM	23	23	2.99 (1.80, 4.97)		0.0001*	
	5-IM	29	29	2.19 (1.53, 3.13)		0.0094*	
	4-IM	34	34	1.92 (1.36, 2.72)		0.0392*	
	C	37	37	1.04 (0.88, 1.23)			
43	8-SQ	29	29	2.51 (1.90, 3.33)	<0.0001*	0.0004*	
	8-IM	32	32	2.26 (1.72, 2.98)		0.0019*	
	7-IM	23	23	3.3 (2.05, 5.30)		<0.0001*	
	5-IM	30	30	2.9 (2.09, 4.02)		<0.0001*	
	4-IM	33	33	1.79 (1.26, 2.54)		0.0703	
	C	33	33	1.03 (0.94, 1.13)			

Human T-Cell Proliferation for ATP Correlates of Protection Subjects: Stimulation Index Geometric Means and Confidence Intervals

C = Control group

Cells contain all pairwise comparisons between Groups that are significant at the 0.05 level. The format within each cell is: (1) the relationship between corresponding arm means and (2) the Tukey-adjusted p-value.

* Statistically significant at the 0.05 level of significance.

TABLE S10. Time points with significant increases in PA-induced peripheral blood mononuclear cells cytokine mRNA levels

	Cytokine																														
	IFN-γ					IL-2					IL-4					IL-6					IL-1β					TNF-α				
Time Point (Months)	8-SQ	8-IM	7-IM	5-IM	4-IM	8-SQ	8-IM	7-IM	5-IM	4-IM	8-SQ	8-IM	7-IM	5-IM	4-IM	8-SQ	8-IM	7-IM	5-IM	4-IM	8-SQ	8-IM	7-IM	5-IM	4-IM	8-SQ	8-IM	7-IM	5-IM	4-IM
0																														
0.5														+																
1						+	+				+	+		+	+						+				+	+			
2																										+				
6								+					+	+									+					+		
7						+	+	+	+	+								+			+	+	+	+	+	+	+	+	+	+
12						+		+	+	+					+			+	+	+			+	+	+			+	+	+
13			+					+		+			+	+	+			+			+	+	+		+	+	+	+		
18							+	+	+	+							+	+										+		
19			+				+	+	+	+			+					+				+	+		+	+	+	+	+	+

TABLE S11. PA-specific memory B cell frequencies by study group ATP

Month	Group	Number of Observations/ group (N)	N>0	Frequency (% rPA specific IgG Secreting Cells)	Standard Error	Group Effect P-value	Tukey Adjusted P-value for Significant Differences from the Controls (C)	Significant Differences Between the Groups (Relationship) Tukey's P-value #
0	8-SQ 8-IM 754-IM C	7 7 22 8	0 0 1 0	0 0 0 0	0 0 0 0	0.8127	1.0000 1.0000 0.8915	
0.5	8-SQ 8-IM 754-IM C	6 5 15 4	1 1 5 2	0 0.02 0.01 0.04	0 0.02 0 0.03	0.2173	0.2529 0.7538 0.2305	
1	8-SQ 8-IM 754-IM C	6 7 18 6	3 2 1 3	0.01 0.03 0 0.02	0 0.03 0 0.01	0.1021	0.8740 0.7853 0.6112	
2	8-SQ 8-IM 754-IM C	7 5 23 6	3 2 7 1	0.01 0 0.01 0.01	0.01 0 0.01 0.01	0.9529	0.9996 0.9721 1.0000	
6	8-SQ 8-IM 754-IM C	6 8 22 7	4 8 16 2	0.02 0.11 0.04 0.03	0.01 0.03 0.01 0.03	0.0072*	0.9982 0.0286* 0.9918	8-SQ<8-IM, 0.0251 8-IM>754-IM, 0.0092
7	8-SQ 8-IM 754-IM C	36 38 116 42	36 38 105 7	0.15 0.22 0.14 0.01	0.02 0.03 0.01 0.01	<0.0001*	0.0003* <0.0001* <0.0001*	8-IM>754-IM, 0.019
12	8-SQ 8-IM 754-IM C	6 8 19 9	6 7 17 2	0.08 0.16 0.12 0	0.02 0.04 0.03 0	0.0067*	0.4480 0.0073* 0.0180*	
13	8-SQ 8-IM 7-IM 54-IM C	5 6 5 10 7	5 6 5 10 .	0.2 0.21 0.13 0.17 0	0.07 0.09 0.03 0.05 0	0.0924	0.1675 0.1044 0.5792 0.1777	

Month	Group	Number of Observations/group (N)	N>0	Frequency (% rPA specific IgG Secreting Cells)	Standard Error	Group Effect P-value	Tukey Adjusted P-value for Significant Differences from the Controls (C)	Significant Differences Between the Groups (Relationship) Tukey's P-value #
18	8-SQ	34	29	0.08	0.02	<0.0001*	0.0024*	
	8-IM	27	26	0.13	0.03		<0.0001*	
	7-IM	28	24	0.08	0.01		0.0055*	
	54-IM	53	45	0.07	0.01		0.0072*	
	C	34	6	0	0			
19	8-SQ	5	5	0.14	0.04	0.1240	0.1862	
	8-IM	3	3	0.16	0.08		0.1915	
	7-IM	5	5	0.12	0.04		0.3020	
	5-IM	2	2	0.09	0.02		0.8552	
	4-IM	6	5	0.09	0.06		0.5947	
	C	8	2	0	0			
30	8-SQ	6	6	0.12	0.02	0.0050*	0.7002	(8-IM>4-IM) 0.0044
	8-IM	6	6	0.25	0.09		0.0086*	
	7-IM	9	9	0.09	0.02		0.8336	
	5-IM	3	2	0.04	0.02		1.0000	
	4-IM	8	4	0.03	0.02		1.0000	
	C	6	4	0.03	0.02			
31	8-SQ	6	6	0.2	0.09	0.0629	0.2892	
	8-IM	5	5	0.19	0.08		0.3756	
	7-IM	7	7	0.22	0.08		0.1607	
	5-IM	1	0	0	--		1.0000	
	4-IM	8	5	0.03	0.02		0.9995	
	C	6	1	0.01	0.01			
42	8-SQ	24	16	0.14	0.04	0.0349*	0.3525	
	8-IM	24	21	0.21	0.04		0.0115*	
	7-IM	21	15	0.09	0.04		0.9394	
	5-IM	21	14	0.11	0.03		0.7700	
	4-IM	26	18	0.12	0.04		0.5801	
	C	33	18	0.05	0.02			
43	8-SQ	8	6	0.17	0.05	0.1493	0.4840	
	8-IM	6	5	0.21	0.09		0.3111	
	7-IM	2	2	0.35	0.15		0.1421	
	5-IM	4	4	0.19	0.13		0.5280	
	4-IM	5	5	0.09	0.02		0.9461	
	C	5	1	0	0			

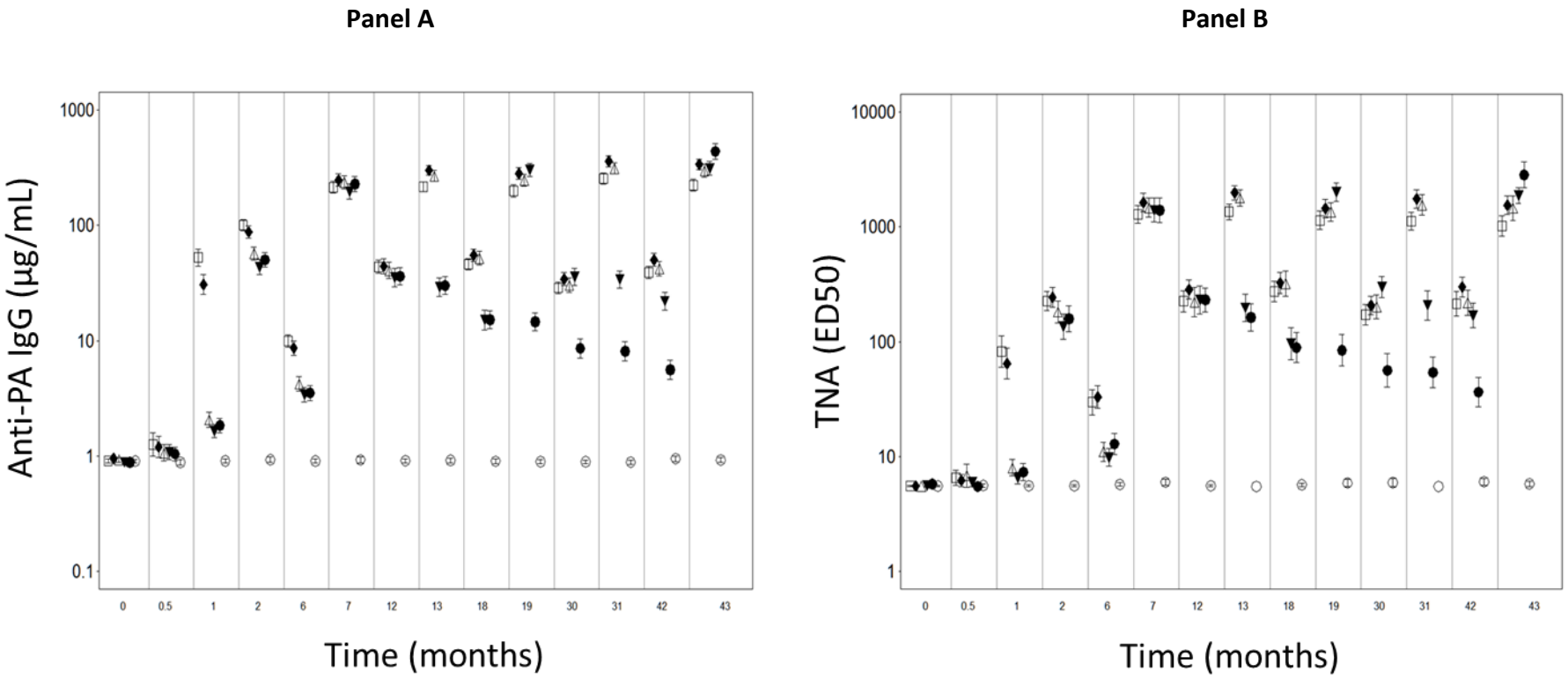
Memory B cell frequencies (% rPA specific cells per total IgG secreting cells) in PBMCs were analyzed with outliers excluded. Data from study groups 4-IM, 5-IM, and 7-IM (Table S2) were combined for analysis (754-IM) up to the Month 12 time point; data from study groups 4-IM and 5-IM were combined (54-IM) for analysis of the Months 13 and 18 time points. For visual inspection, only groups with N ≥5 were graphed (Figure S3).

Cells contain all pairwise comparisons between Groups that are significant at the 0.05 level. The format within each cell is: (1) the relationship between corresponding group means and (2) the Tukey-adjusted p-value.

* Statistically significant at the 0.05 level.

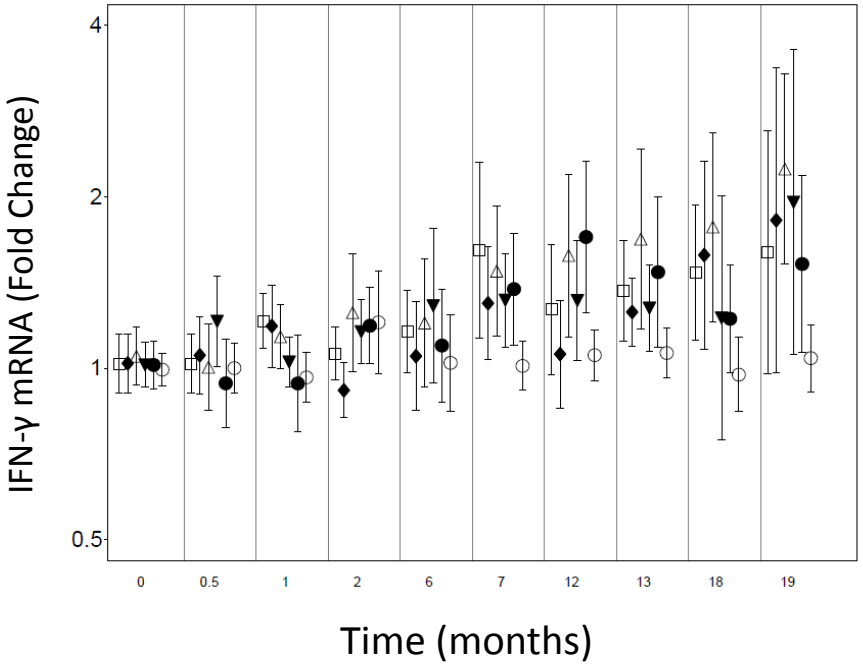
-- Standard Error was not calculated, as only one value was available.

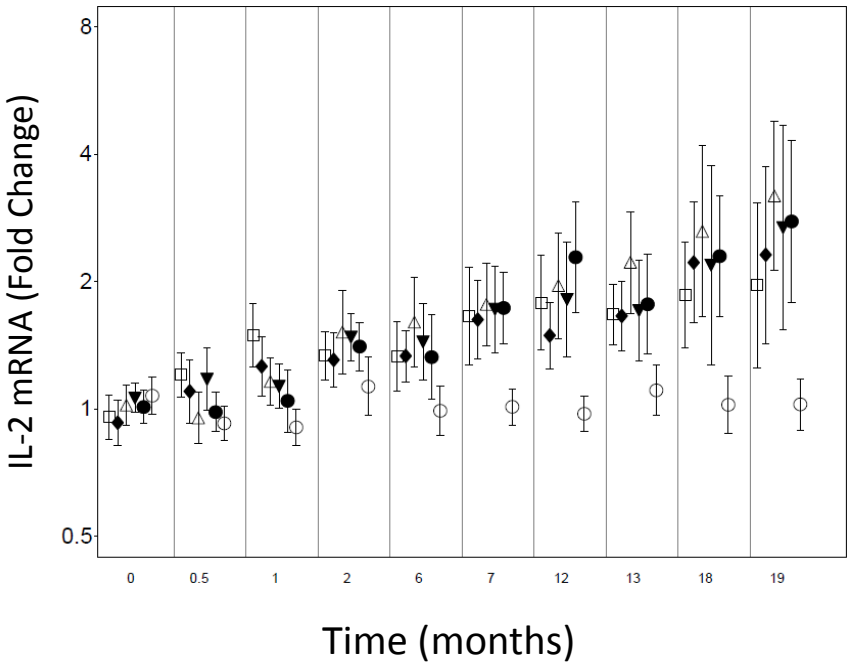
FIG S1. Humoral anti-PA IgG antibody responses to the annual and reduced frequency prime-boost schedules of AVA in humans.



Serum anti-PA IgG geometric mean concentrations (GMC, µg/mL) (***Panel A***) and lethal toxin neutralization activity (TNA) (GMT, ED50) (***Panel B***) for each study group over time (Month s). Error bars represent the 95%CI. Geometric means were calculated after masking values below the LOD as ½ LOD. LOD for anti-PA IgG was 1.7 µg/mL, LOD for TNA was 11.
☐ – 8-SQ; ◆ – 8-IM; △ – 7-IM; ▼ – 5-IM; ● – 4-IM; ○ – Controls.

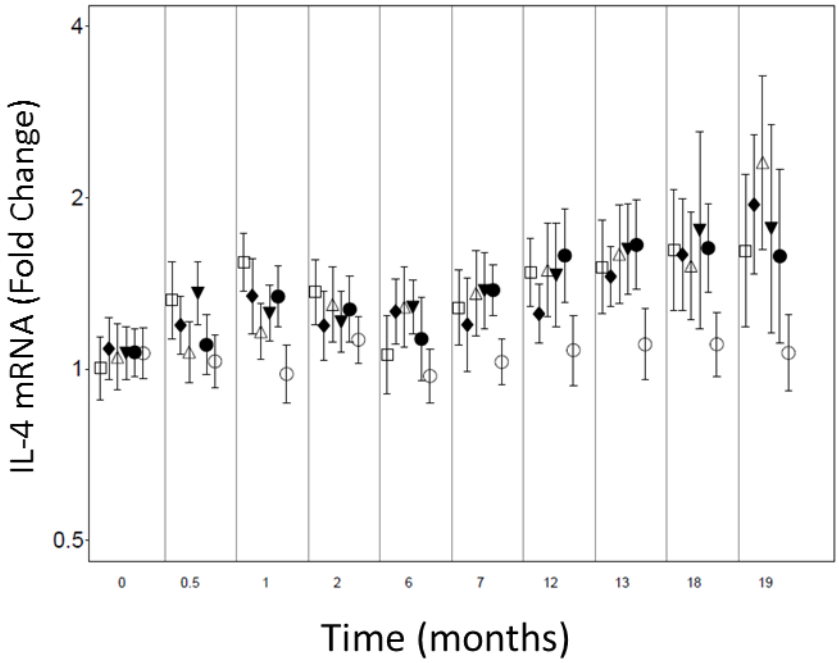
FIG S2A. Transcription profiles for Th1 cytokines IFN-γ and IL-2 in humans vaccinated with AVA.

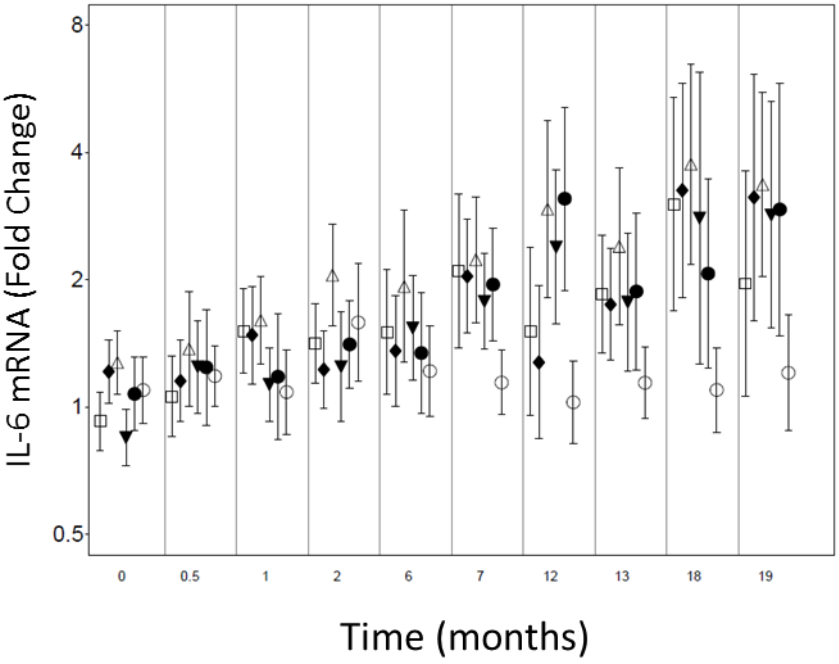




Changes in IFN-γ and IL-2 mRNA levels as determined by RT-qPCR were interpreted as representing Th1-type responses. Similar to the NHPs, the human cytokine responses demonstrated both Th1 and Th2 responses with evidence of increased gene expression to all cytokines at selected time points for all schedules.
☐ - 8-SQ; ◆ - 8-IM; △ - 7-IM; ▼ - 5-IM; ● - 4-IM; ○ – Controls.

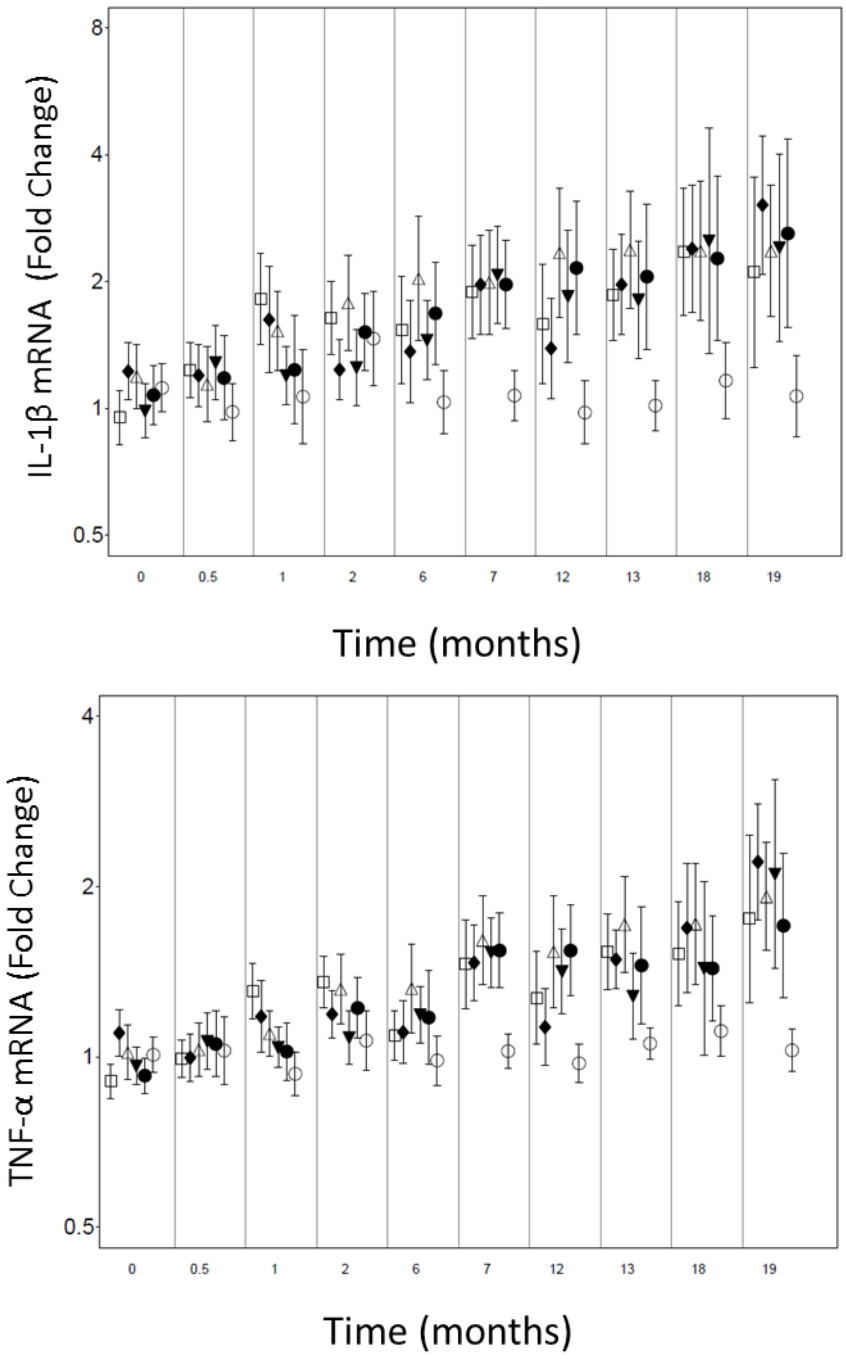
FIG S2B. Transcription profiles for Th2 cytokines IL-4 and IL-6 in humans vaccinated with AVA.





Changes in IL-4 and IL-6 mRNA levels as determined by RT-qPCR were interpreted as representing Th2-type responses. IL-6, an important accessory cell cytokine, was interpreted as representing T-cell activation. Similar to the NHPs, the human cytokine responses demonstrated both Th1 and Th2 responses with evidence of increased gene expression to all cytokines at selected time points for all schedules.
☐ - 8-SQ; ◆ - 8-IM; △ - 7-IM; ▼ - 5-IM; ● - 4-IM; ○ – Controls.

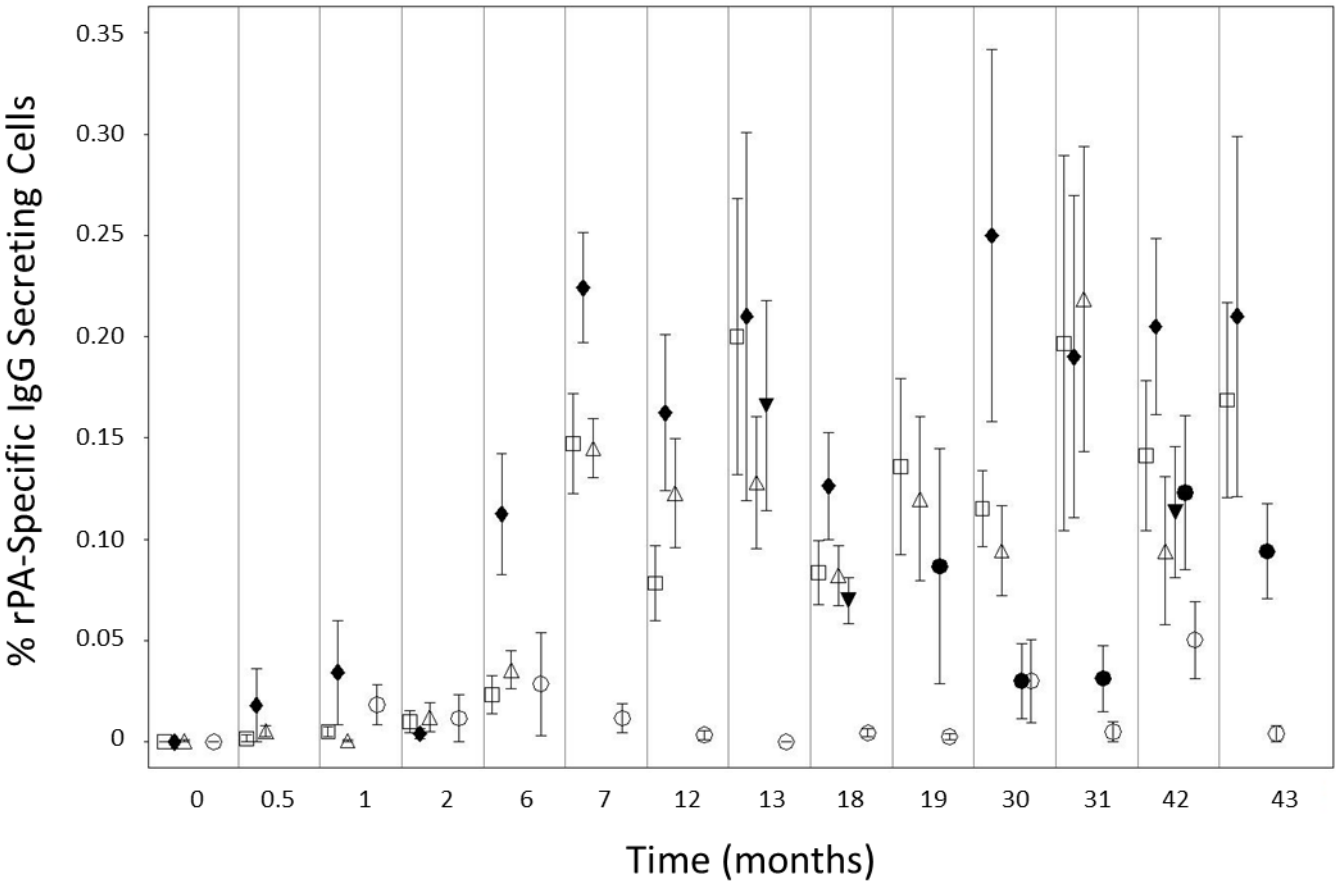
FIG S2C. Transcription profiles for acute phase cytokines IL-1β and TNF-α in humans vaccinated with AVA.



Changes in IL-1β and TNF-α mRNA levels as determined by RT-qPCR were interpreted as representative of acute phase immune responses. Similar to the NHPs, the human cytokine responses demonstrated both Th1 and Th2 responses with evidence of increased gene expression to all cytokines at selected time points for all schedules.

☐ - 8-SQ; ◆ - 8-IM; △ - 7-IM; ▼ - 5-IM; ● - 4-IM; ○ – Controls.

FIG S3. PA-specific B cell frequencies in humans vaccinated with AVA.



Memory B cell frequencies (% rPA specific cells per total IgG secreting cells) in PBMCs were analyzed with outliers excluded. Data from study groups 4-IM, 5-IM, and 7-IM were combined for analysis (754-IM) up to the Month 12 time point; data from study groups 4-IM and 5-IM were combined (54-IM) for analysis of the Months 13 and 18 time points. For visual inspection, only groups with N≥5 were graphed. Error bars indicate 1 standard error (SE). Data, including groups and time points with N<5, are presented in Table S11.

□ - 8-SQ; ◆ - 8-IM; △ - 7-IM and 754-IM to Month 12; ▼ - 5-IM and 54-IM at Months 13 and 18; ● - 4-IM; ○ – Controls.

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